MS06-2-12 Role of flexible domain II in the homotropic cooperativity of Aspergillus niger glutamate dehydrogenase
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Abstract
Homotropic cooperativity is a part of allosteric mechanism mostly observed in the multimeric proteins such as haemoglobin, ATCase, etc. (1). The atomic details on inter-subunit communications during the homotropic cooperativity are scanty. Thus we attempt to understand this fundamental phenomenon in an important metabolic enzyme, glutamate dehydrogenase (GDH). It reversibly catalyzes the reductive amination of α-ketoglutarate to L-glutamate in presence of ammonia and the coenzyme NAD(P)H, and thus interlinks the carbon and nitrogen cycles (2). The hexameric NADP-specific GDHs from *Aspergillus niger* (AnGDH) and *Aspergillus terreus* (AtGDH) share 88% sequence identity. Interestingly, AnGDH shows homotropic cooperativity towards its substrate α-ketoglutarate (n_H = 3.0) while AtGDH shows Michaelian kinetics (3). Therefore, structural analysis of AnGDH and AtGDH will help to unravel the mechanistic details about the homotropic cooperativity. In that context, we have recently solved the crystal structures of AtGDH as dimer in the asymmetric unit, which generates into a biologically functional hexamer on applying the three fold crystallographic symmetry operation. There are two different conformational states in each dimer – open (~17 Å) and partial closed state (~13 Å). Each subunit of AtGDH consists of two domains -- rigid domain I for substrate binding and hexamerization, and flexible domain II involved in coenzyme binding. Structural comparison between AtGDH and AnGDH (5XVI) shows differential domain II dynamics. In AnGDH (5XVI) the maximal mouth opening distance measured between the cleft residues Lys122 (domain I) and Arg282 (domain II) is ~21 Å, and in AtGDH the same is ~17 Å. The amino acid substitutions in domain II probably regulate the extent of mouth openings in these two enzymes. Based on the structural analysis, twelve mutants of AtGDH have been generated sequentially on *Atgdh* gene background and their responses to α-ketoglutarate substrate saturation of the purified mutants were performed. Further the kinetic studies on AtGDH sequential mutants (Ala220Lys, Arg246Ser and Ile433Val located in the domain II) showed changes in the substrate affinity (K_m) and maximum reaction velocity (V_{max}). Thus, our findings suggest that the extent of mouth opening might regulates the rate of enzyme catalysis, and thus ultimately likely to be linked to the homotropic cooperativity. The findings of our study would help in understanding the homotropic cooperativity in hexameric GDHs from lower organisms.

References
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Dynamics of domain II in hexameric AtGDH